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Comparative effects of crop residue incorporation and inorganic potassium fertilization on soil C and N characteristics and microbial activities in cotton field

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Abstract

Background: Crop residue incorporation into the soil is an effective method to augment soil potassium (K) content, and effects of crop residue and K fertilizer on soil K balance have been compared. However, their influences on other soil characteristics such as carbon (C) and nitrogen (N) characteristics and microbial activities have not been quantified. To address this, field experiments were conducted in 2011 at Dafeng (sandy loam) and Nanjing (clay loam) in China with treatments including blank control without crop residue incorporation and K fertilizer application, 0.9 t·ha^{-1} wheat straw incorporation (W1C0), 0.7 t·ha^{-1} cotton residue incorporation (W0C1), 0.9 t·ha^{-1} wheat straw + 0.7 t·ha^{-1} cotton residue incorporation (W1C1) and two K fertilizer rates (150 and 300 kg·ha^{-1}(K_2O)) during the cotton season.

Results: Compared with control, K fertilizer treatments did not alter soil water-soluble organic carbon/soil organic carbon (WSOC/SOC) ratio, microbial biomass carbon (MBC)/SOC ratio, MBC/microbial biomass nitrogen (MBN) ratio, water inorganic nitrogen/total nitrogen ratio (WIN/TN), the number of cellulose-decomposing bacteria, or related enzymes activities, however, W0C1, W1C0 and W1C1 treatments significantly increased WSOC/SOC ratio, MBC/SOC ratio and MBC/ MBN ratio, and decreased WIN/TN ratio at both sites. W0C1, W1C0 and W1C1 treatments also increased the number of soil cellulose-decomposing bacteria and activities of cellulase, β-glucosidase and arylamidase. Regarding different crop residue treatments, W1C0 and W1C1 treatments had more significant influences on above mentioned parameters than W0C1 treatment. Moreover, MBC/MBN ratio was the most important factor to result in the differences in the number of cellulose-decomposing bacteria and soil enzymes activities among different treatments.

Conclusions: Short-term K fertilizer application doesn't affect soil C and N availability and microbial activities. However, crop residue incorporation alters soil C and N characteristics and microbial activities, and the influence of wheat straw is much stronger than that of cotton straw.

Keywords: Crop straw, Potash fertilizer, Soil quality

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Background

High-yield crop varieties, which need more nutrients including potassium (K) to maintain growth and development compared with traditional crop varieties, have been widely used worldwidely, which will absorb large amounts of K from soil, resulting in soil K deficiency (Jin 1997; Wang et al. 2008). Thus, a large amount of chemical K fertilizer is applied every year to keep soil K balance, resulting in that the price of K fertilizer are gradually rising (Schloter et al. 2003). The replacement of chemical K fertilizer by other materials and the decrease of K fertilizer application amount have been hot research fields (Zörb et al. 2014). With the increasing of crop yield, more and more crop residues are produced. As a recycled organic resource, crop residues contain abundant carbon (C), nitrogen (N), phosphorus (P) and K. Demonstrably, more than 90% K content reserved in crop straw can be released during the first 30 days of decomposition (Sui et al. 2017). Therefore, crop straw incorporation can be an effective method to improve soil K content and this method have been used in many parts of the world (Yadvinder-Singh et al. 2004; Sui et al. 2017).

Soil characteristics are closely related to crop yield and quality (Zhou et al. 2007; Tittonell et al. 2012; Wang et al. 2012). Soil characteristics generally contain soil nutrients contents, microorganisms, microbial biomass, enzyme activities, etc. (Islam and Weil 2000; Schloter et al. 2003; Paz-Ferreiro and Fu 2016). It is reported that soil nutrients contents can influence soil microorganisms (Cheshire and Chapman 1996; Mueller et al. 1998; Martens 2000; Tu et al. 2006). Generally, the ratio of C/N was very important for soil microorganisms and soil microbes are C-limited (Smith et al. 1990). Organic matter quantity and quality applied to the soil become the most important factors impacting microbial community structure and microbial biomass (Wardle 1992; Fließbach and Mäder 2000). A higher easily decomposable organic C content is conducive to the rapid growth of soil microorganisms, easily leading to higher microbial biomass and soil enzymes activities. For example, Chowdhury et al. (2000) observed that compared with rice husk and sawdust composts, a manure compost had high easily decomposable C, which was more effective to enhance soil microbial biomass C. Moreover, soil enzymes activities are closely associated with the rate of microbial mediated processes, and the diversity of enzyme is closely related to the complexity of soil organic matter (Paz-Ferreiro and Fu 2016). Thus, crop straw incorporated in soil can bring a large amount of organic matter and C, which will influence soil characteristics (Yadvinder-Singh et al. 2004; Sui et al. 2015).

Double cropping systems have been used in many countries (Heggenstaller et al. 2008; Graß et al. 2013; Sui et al. 2015). For example, a wheat-cotton rotation system is widely used in the Yangtze River in China, and compared with a single system, two different kinds of straw are produced in double cropping systems (Sui et al. 2015). In production, wheat straw and cotton residue are produced about 0.9 and 0.7 $t{\cdot}ha^{-1}$ per year, respectively, in Yangtze River Valley. Recently, Sui et al. (2015) and Yu et al. (2016) found that for the wheat-cotton rotation system, in the first and second year, 0.9 $t{\cdot}ha^{-1}$ wheat straw or 0.7 $t{\cdot}ha^{-1}$ cotton residue incorporation in soil before planting cotton could replace 150 $kg{\cdot}ha^{-1}$ of inorganic K fertilizer for cotton growth. Then, they compared the influences of wheat straw, cotton straws and chemical K fertilizer on soil apparent K balance. However, apart from soil K nutrient, soil characteristics also contain soil C and N status, microbial content, enzyme activities, etc., which can be influenced by organic or inorganic fertilizer and soil environment. For example, Belay et al. (2002) reported that long-term K fertilization altered total organic C, basic cation contents, microbial biomass and numbers of fungi, bacteria and actinomycetes in soil; Yadvinder-Singh et al. (2004) reported that long-term crop straw incorporation could alter the soil environment, which can influence soil microorganisms and enzymes activities. Although Yu et al. (2016) and Sui et al. (2017) have studied and compared the impacts of wheat straw, cotton straw and chemical fertilizer on soil K balance, the effects of wheat straw, cotton straw and K fertilizer on other characteristics of soil have not been studied.

Based on previous findings that wheat and cotton straw could completely replace K fertilizer for cotton growth (Sui et al. 2015; Yu et al. 2016; Sui et al. 2017), it is hypothesized that wheat straw and cotton straw have similar effects to K fertilizer on other characteristics of soil. Therefore, the objective of this study was to explore and compare the impacts of wheat residue input, cotton residue input and K fertilizer on soil C and N characteristics (such as WSOC/SOC, MBC/SOC, WIN/TN, MBN/TN etc.) and soil microbial activities (such as the number of bacteria and activities of enzymes) during different growth stages of cotton.

Materials and methods

Experimental sites

A field experiment was carried out during the cotton season of 2011, at two sites simultaneously. The first site was at the Jiangsu Academy of Agricultural Sciences in Nanjing (32°20′ N and 118°52′ E), and the second site was at the Dafeng Basic Seed Farm in Dafeng (33°24′ N and 120°34′ E), Jiangsu province. Both locations are situated on the downstream sections of the Yangtze River in China. The types of soil in Dafeng and Nanjing were sandy loam (49.0% silt, 29.5% clay and 21.5% sand) and clay loam (36.2% silt, 6.8% clay and 57.0% sand),

respectively. The top soil of 0–20 cm for the experimental field in Dafeng and Nanjing had the following properties before cotton transplanting: 1.44 and 1.32 $g \cdot cm^{-3}$ bulk density, pH 7.9 and 5.7, 12.1 and 9.5 $g \cdot kg^{-1}$ soil organic carbon (SOC), 1.18 and 0.90 $g \cdot kg^{-1}$ total N (TN), 26.4 and 24.2 $mg \cdot kg^{-1}$ water inorganic N (WIN), 22.2 and 15.1 $mg \cdot kg^{-1}$ Olsen-P, 18.4 and 16.5 $g \cdot kg^{-1}$ structural K, 60.6 and 20.5 $mg \cdot kg^{-1}$ water-soluble K, 255.8 and 134.1 $mg \cdot kg^{-1}$ exchangeable K, 1.1 and 0.6 $g \cdot kg^{-1}$ non-exchangeable K, respectively. Both sites experience subtropical monsoon climates. Daily temperature and precipitation during cotton growth stage for the two experimental sites were showed in Fig. 1.

Experimental design

Cotton (cv. Siza 3) seeds were planted on April 25th in a nursery. After the wheat harvest on May 31th, cotton seedlings were transplanted to the fields on June 1st with a row spacing of 100 cm and a plant spacing of 30 cm. The plant density was 33 400 plants·ha^{-1}.

Sui et al. (2015) and Yu et al. (2016) found that in the first and second year, 0.9 t·ha^{-1} wheat straw or 0.7 t·ha^{-1} cotton residue incorporation in soil before planting cotton could replace 150 kg·ha^{-1} of inorganic K fertilizer for cotton growth. Therefore, wheat straw at rates of 0 and 0.9 t·ha^{-1} (W0 and W1) and cotton residue at rates of 0 and 0.7 t·ha^{-1} (C0 and C1) were applied. In addition, two K fertilizer treatments at 150 and 300 kg·ha^{-1} of K_2O were optimal and abundant K application rates, respectively, for cotton growth in the Yangtze River Valley (Hu et al. 2015). Consequently, there were six treatments in this experiment: neither crop residue incorporation nor K fertilizer application (control), 0.9 t·ha^{-1} wheat straw alone (W1C0), 0.7 t·ha^{-1} cotton residue alone (W0C1), 0.9 t·ha^{-1} wheat straw + 0.7 t·ha^{-1} cotton residue (W1C1), 150 kg·ha^{-1} of K_2O without crop residue incorporation (K150) and 300 kg·ha^{-1} of K_2O without crop residue incorporation (K300). The amount of N and P fertilizer applied in all treatments were adequate for cotton growth, with 300 kg·ha^{-1} (N) and 150 kg·ha^{-1}(P_2O_5). A complete randomized

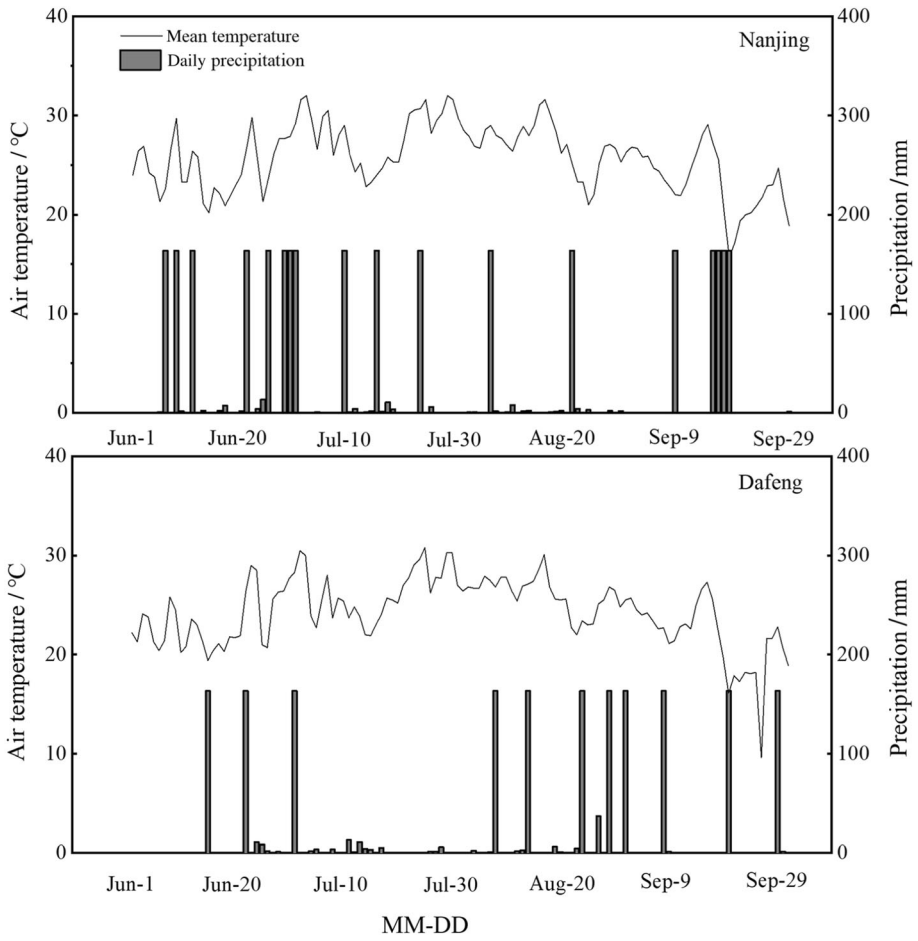

Fig. 1 Daily temperature and precipitation during cotton growth stage at Nanjing Experimental Station and Dafeng Experimental Station in 2011. All data were collected from the Weather Station located in each experimental site

block design was used with three replicates in each experimental location. Other crop managements including weed and pest control were performed according to local practices.

In addition, the wheat straw including stems, leaves, and chaff was applied to the soil and the cotton residue including roots, stem-branches, leaves, and carpels was applied. Crop residues were smashed and mixed prior to being incorporated into the top soil (0–20 cm) of the experiment plots.

Soil sampling

Six randomized soil cores (3 cm in diameter) were sampled per plot with a hand auger from 0 to 20 cm depth, at cotton seedling stage (15-June), flowering stage (15-July), boll-setting stage (15-August) and boll-opening stage (15-September) in 2011 at both experimental sites. The soil samples were sieved using a 2 mm mesh and then were stored in a 4 °C refrigerator for subsequent determination.

Laboratory analysis

The contents of microbial biomass C (MBC) and N (MBN) were measured according to Griffiths et al. (2012). Dry soil (10 g) was fumigated at 25 °C for 24 h and extracted with 0.5 $mol{\cdot}L^{-1}$ K_2SO_4. In the extracts, total organic C was measured by combustion with a Shimadzu TOC-VCPH analyser; total organic N was measured by alkaline persulfate oxidation. Soil microbial biomass C and N contents were calculated as the difference between the fumigated and unfumigated samples using equal conversion factors of 0.45 for C and N. Water-soluble organic carbon content (WSOC) was determined using a total organic carbon analyzer (Shimadzu, 5000A) according to Yang et al. (2003). Soil water inorganic N was determined as described by Fan et al. (2005). Moist soil sub-sample (12 g) was extracted by shaking with 100 mL if 0.01 $mol{\cdot}L^{-1}$ $CaCl_2$ for 12 h. The extracts were used for N content analysis by a continuous flow analyzer (TRAACS Model 2000 analyzer). The concentration of TN was assayed using the Kjeldahl method, and the concentration of SOC was analyzed by dichromate digestion (Lu 2000).

The number of soil cellulose-decomposing bacteria was analyzed according to Zuo et al. (2014). 90 mL distilled water and 10 g soil sample were placed into a 500 mL conical flask before shaking for 10 min. The obtained solution was diluted up to 1 million-fold with sterile distilled water. Next, 1 mL solution was poured into 50 mL cellulose congo red medium before incubating for 4 days at 30 °C. Counts were made after the microbe communities were established. The result was expressed as colony-forming units (CFU) per gram of dry soil.

Arylamidase (EC 3.4.11.2) activity was measured according to Tabatabai et al. (2002). 1 mL of 8.0 $mmol{\cdot}L^{-1}$ l-leucine β-naphthylamide hydrochloride, 3 mL of 0.1 $mol{\cdot}L^{-1}$ tris-aminomethane buffer and 1 g soil were incubated at 37 °C for 1 h. 6 mL of ethanol (95%) was added to stop the reaction before centrifuging at 12 000 *g* for 2 min. Then 1 mL supernatant, 2 mL acidified ethanol, 1 mL ethanol, and 2 mL p-dimethylamin ocinnamaldehyde reagent were mixed before measuring the absorbance at 540 nm. β-glucosidase (EC 3.2.1.21) activity was assayed as described by Tabatabai (2002). Soil sample (1 g), 5 $mmol{\cdot}L^{-1}$ p-nitrophenyl β-d-glucoside (1 mL) and modified universal buffer (4 mL) were incubated for 1 h at 37 °C. 4 mL of 0.1 $mol{\cdot}L^{-1}$ tris-aminomethane and 1 mL of 0.5 $mol{\cdot}L^{-1}$ $CaCl_2$ were added to stop the reaction. Then, the mixture was centrifuged at 12 000 *g* for 2 min before measuring the absorbance at 412 nm. For the measurement of cellulase (EC 3.2.1.4) activity, 5 g soil sample and five-tenths of a milliliter of toluene were placed into a 50 mL flask. After 15 min, 10 mL acetate buffer at pH 5.9 and 10 mL of 1% carboxy methyl cellulose were added before incubating at 30 °C for 24 h. Then, 50 mL distilled water was added before filtering through Whatman 30 filter paper. The filtrate was made up to 100 mL using distilled water. The Nelson's method was used to measure the reducing sugar content in the filtrate (Pancholy and Rice 1973).

Statistical analysis

The variance analysis was performed by SPSS 20.0. The comparison of means was made using the least significant difference (LSD) at the 0.05 probability level. Different letters in the tables indicate statistically significant differences at $P < 0.05$.

The data of the amount of cellulose-decomposing bacteria and activities of cellulase, β-glucosidase and arylamidase and soil WSOC/SOC, MBC/SOC, WIN/TN, MBN/TN and MBC/MBN ratios were analyzed using the mixed model fitted by restricted maximum likelihood. The amount of cellulose-decomposing bacteria and activities of cellulase, β-glucosidase and arylamidase were dependent variables, respectively. WSOC/SOC, MBC/SOC, WIN/TN, MBN/TN and MBC/MBN ratios were fixed effects. Calculations were done with the mixed procedure in the SAS system.

Results

Soil carbon and nitrogen nutrients

This experiment was conducted in order to compare the effects of different treatments on soil C and N changes and soil microbial activities. Although the experiment was conducted at two different sites, all the measured

Table 1 Results of ANOVA (Analysis of variance) on the effects of experimental site (St), cotton growth stage (S), treatment (T) and their interactions on WSOC/SOC, MBC/SOC, WIN/TN, MBN/TN and MBC/MBN ratios, cellulose-decomposing bacteria amounts (CDBA), cellulase (CE), β-glucosidase (β-GE) and arylamidase (AE) activities

	WSOC/SOC	MBC/SOC	WIN/TN	MBN/TN	MBC/MBN	CBDA	CE	β-GE	AE	*df*
St	15.98**	119.48**	4.05*	61.65**	0.61	388.88**	875.62**	95.28**	865.47**	1
S	791.17**	709.10**	1541.43**	736.55**	67.92**	378.73**	378.73**	358.17**	893.83**	3
T	70.49**	31.79**	73.18**	22.75**	44.39**	75.21**	18.83**	71.43**	26.02**	5
St × S	29.35**	41.08**	38.42**	25.22**	2.78*	0.22	2.85*	9.19**	18.99**	3
St × T	0.49	1.75	1.36	1.22	1.68	1.14	0.13	0.40	0.82	5
S × T	5.63**	1.95*	5.75**	4.07**	4.85**	1.96*	1.53	3.64**	3.27**	15
St × S × T	0.61	0.49	0.70	0.68	0.74	1.32	0.27	1.08	1.57	15

WSOC, water-soluble organic carbon content; SOC, soil organic carbon; MBC, microbial biomass carbon; WIN, water inorganic nitrogen; TN, total nitrogen; MBN, microbial biomass nitrogen; MBC, microbial biomass carbon
F values and significance levels (**$P < 0.01$, *$P < 0.05$)

parameters were not affected by experimental site × cotton stage × treatment or experimental site × treatment (Table 1), indicating that the effects of different treatments on all the measured characteristics of soil were similar for the two sites. Thus, this paper focused on the interaction of cotton stage × treatment and their main effects.

Soil WSOC/SOC ratio and MBC/SOC ratio were significantly affected by growth stages × treatment ($P < 0.01$, Table 1). W0C1 and K fertilizer treatments (K150 and K300) have no significant influences on WSOC/SOC ratio and MBC/SOC ratio compared with control ($P > 0.05$, Tables 2 and 3). W1C0 treatment did not alter MBC/SOC ratio, but had a higher WSOC/SOC ratio than control at boll-setting and boll-opening stages at both sites. Additionally, WSOC/SOC ratio and MBC/SOC ratio in W1C1 treatment were the highest at all growth stages at both sites (Tables 2 and 3).

Soil WIN/TN ratio and MBN/TN ratio were significantly affected by the interaction between treatment and growth stage ($P < 0.05$, Table 1). No significant differences in soil WIN/TN ratio or MBN/TN ratio between K fertilizer treatments and control were measured at any stages at both sites ($P > 0.05$, Tables 4 and 5). However, compared with control, WIN/TN ratio was lower in W0C1 treatment at seedling stage, in W1C0 treatment at flowering, boll-setting and boll-opening stages, and in W1C1 treatment at all four growth stages at both sites. MBN/TN ratio in W1C0 treatment was lower than that in control at boll-setting stage and MBN/TN ratio in W1C1 treatment was lower than that in control at flowering stage at both sites.

MBC/MBN ratio was significantly affected by the interaction between treatment and cotton growth stage ($P < 0.01$, Tables 1 and 6). There were no significant differences between K fertilizer treatments and control at any stages at both sites ($P > 0.05$, Table 6). Compared with control, MBC/MBN ratio was higher in W0C1 treatment at seedling stage, in W1C0 treatment at flowering and boll-setting stages, and in W1C1 treatment at seedling, flowering and boll-setting stages at both sites.

Table 2 Effects of crop residue incorporation and K fertilization on soil WSOC/SOC ratio (%)

Treatment	Nanjing				Dafeng			
	SS	FS	BS	BOS	SS	FS	BS	BOS
Control	0.36 ± 0.02b	0.50 ± 0.02ab	0.58 ± 0.01c	0.35 ± 0.01c	0.32 ± 0.02c	0.50 ± 0.02ab	0.53 ± 0.02c	0.39 ± 0.02c
W0C1	0.39 ± 0.02b	0.47 ± 0.02bc	0.63 ± 0.03bc	0.37 ± 0.02c	0.35 ± 0.03bc	0.47 ± 0.02b	0.55 ± 0.02bc	0.42 ± 0.01bc
W1C0	0.38 ± 0.00b	0.49 ± 0.02b	0.65 ± 0.03b	0.43 ± 0.03b	0.36 ± 0.02b	0.47 ± 0.03b	0.60 ± 0.03b	0.45 ± 0.03b
W1C1	0.42 ± 0.01a	0.54 ± 0.03a	0.77 ± 0.03a	0.49 ± 0.02a	0.41 ± 0.02a	0.53 ± 0.02a	0.69 ± 0.04a	0.51 ± 0.02a
K150	0.37 ± 0.01b	0.46 ± 0.02bc	0.61 ± 0.04bc	0.37 ± 0.01c	0.33 ± 0.01bc	0.48 ± 0.03b	0.57 ± 0.02bc	0.42 ± 0.02bc
K300	0.37 ± 0.01b	0.44 ± 0.02c	0.62 ± 0.02bc	0.38 ± 0.02c	0.33 ± 0.02bc	0.47 ± 0.03b	0.56 ± 0.02bc	0.41 ± 0.02bc
F value	5.87**	6.27**	17.55**	19.60**	8.89**	3.12*	12.25**	13.56**

WSOC, water-soluble organic carbon content; SOC, soil organic carbon; SS, seedling stage; FS, flowering stage; BS, boll-setting stage; BOS, boll-opening stage. Control, neither crop residue nor K fertilizer; W1C0, 0.9 t·ha^{-1} wheat straw incorporation alone, W0C1, 0.7 t·ha^{-1} cotton straw incorporation alone; W1C1, 0.9 t·ha^{-1} wheat straw incorporation + 0.7 t·ha^{-1} cotton straw incorporation; K150, 150 kg·ha^{-1}of K_2O; K300, 300 kg·ha^{-1} of K_2O
F values and significance levels (**$P < 0.01$, *$P < 0.05$)

Table 3 Effects of crop residue incorporation and K fertilization on soil MBC/SOC ratio (%)

Treatment	Nanjing				Dafeng			
	SS	FS	BS	BOS	SS	FS	BS	BOS
Control	2.95 ± 0.20b	3.94 ± 0.13b	4.96 ± 0.18b	3.45 ± 0.17b	3.47 ± 0.18b	4.07 ± 0.11b	4.77 ± 0.24b	4.06 ± 0.20b
W0C1	3.01 ± 0.13b	3.99 ± 0.25b	5.02 ± 0.22b	3.63 ± 0.14b	3.68 ± 0.18ab	4.31 ± 0.11a	5.05 ± 0.14b	4.09 ± 0.06b
W1C0	3.01 ± 0.15b	4.08 ± 0.08ab	5.02 ± 0.23b	3.57 ± 0.16b	3.52 ± 0.06b	4.36 ± 0.10a	4.87 ± 0.15b	4.26 ± 0.08ab
W1C1	3.34 ± 0.20a	4.36 ± 0.25a	5.87 ± 0.25a	4.15 ± 0.11a	3.86 ± 0.11a	4.49 ± 0.13a	5.44 ± 0.11a	4.49 ± 0.20a
K150	3.01 ± 0.15b	3.98 ± 0.15b	5.04 ± 0.17b	3.50 ± 0.17b	3.57 ± 0.06b	4.27 ± 0.11ab	4.91 ± 0.18b	4.20 ± 0.09b
K300	3.03 ± 0.18b	3.96 ± 0.20b	5.10 ± 0.19b	3.53 ± 0.13b	3.56 ± 0.18b	4.33 ± 0.14a	4.90 ± 0.22b	4.15 ± 0.08b
F value	2.06	2.14	8.23**	8.83**	3.06	4.02*	5.37**	4.12*

MBC, microbial biomass carbon; SOC, soil organic carbon; SS, seedling stage; FS, flowering stage; BS, boll-setting stage; BOS, boll-opening stage. Different treatments are defined in the Table 2
F values and significance levels (**$P < 0.01$, *$P < 0.05$)

Soil bacteria amount and soil enzyme activities

The amount of cellulose-decomposing bacteria was significantly influenced by the interaction between growth stage and treatment ($P < 0.05$, Table 1). Compared with control, W0C1 treatment increased the amount of cellulose-decomposing bacteria by 19.1 and 17.1% at seedling stage at Nanjing and Dafeng experimental sites, respectively (Fig. 2); W1C0 treatment increased the amount of cellulose-decomposing bacteria by 24.5–43.7 and 14.2%–35.0% at boll-setting stage and boll-opening stage, respectively. W1C1 treatment increased the amount of cellulose-decomposing bacteria by 29.8–47.3% and 31.8–51.9% at boll-setting stage and boll-opening stage, respectively. Whereas, there were no significant differences between K fertilizer treatments and control at any stages at both sites.

The activities of cellulase, β-glucosidase and arylamidase were significantly influenced by the interaction between growth stage and treatment ($P < 0.01$, Table 1, except cellulase). Compared with control, W0C1, W1C0 and W1C1 treatments increased cellulase activity by 16.8, 13.3 and 23.2% at Nanjing, and by 9.2, 7.2 and 18.0% at Dafeng at seedling stage (Fig. 3). Additionally, W1C1 treatment also improved cellulase activity by 15.9 and 18.1% against control at boll-setting stage and boll-opening stage at Nanjing, and by 14.8 and 16.1% at Dafeng, respectively. Compared with the control, W0C1, W1C0 and W1C1 treatments significantly increased β-glucosidase activity by 21.3, 16.8 and 32.8% at Nanjing and by 14.0, 15.0 and 27.0% at Dafeng at seedling stage, and by 16.4, 26.1 and 39.2% at Nanjing and by 17.4, 13.9 and 23.5% at Dafeng at boll-setting stage, respectively (Fig. 4). Moreover, W1C1 treatment also significantly increased β-glucosidase activity by 19.3–20.1% over control at flowering stage, and by 21.1–21.7% over control at boll-opening stage. Compared with control, W0C1 treatment had no effective influences on soil arylamidase activity at any stages at both sites, however, W1C0 and W1C1 treatments significantly increased arylamidase activity at seedling, boll-setting and boll-opening stages at both sites ($P < 0.05$, Fig. 5). K fertilizer treatments had no effective influences on all measured soil enzyme activities compared with control at both sites ($P > 0.05$, Figs. 3, 4 and 5).

According to the results of mixed models, the amount of cellulose-decomposing bacteria was significantly influenced by MBC/MBN, MBC/SOC and WSOC/SOC

Table 4 Effects of crop residue incorporation and K fertilization on soil WIN/TN ratio (%)

Treatment	Nanjing				Dafeng			
	SS	FS	BS	BOS	SS	FS	BS	BOS
Control	2.58 ± 0.14a	6.22 ± 0.36a	4.93 ± 0.15a	3.65 ± 0.15a	2.90 ± 0.16a	5.49 ± 0.22a	4.82 ± 0.23a	3.89 ± 0.14a
W0C1	2.24 ± 0.10bc	5.88 ± 0.27a	4.82 ± 0.21a	3.47 ± 0.23a	2.56 ± 0.15b	5.39 ± 0.21a	4.66 ± 0.22ab	3.72 ± 0.15a
W1C0	2.41 ± 0.17ab	5.09 ± 0.21b	4.24 ± 0.18b	3.03 ± 0.11b	2.57 ± 0.13b	4.72 ± 0.17b	4.06 ± 0.17c	3.31 ± 0.10b
W1C1	2.02 ± 0.12c	4.75 ± 0.25b	4.03 ± 0.18b	2.89 ± 0.12b	2.30 ± 0.09c	4.35 ± 0.22b	3.96 ± 0.18c	3.35 ± 0.15b
K150	2.40 ± 0.16ab	5.95 ± 0.34a	4.93 ± 0.29a	3.63 ± 0.18a	2.61 ± 0.05b	5.39 ± 0.29a	4.45 ± 0.21b	3.76 ± 0.21a
K300	2.28 ± 0.13bc	6.32 ± 0.24a	4.94 ± 0.17a	3.78 ± 0.18a	2.71 ± 0.18ab	5.50 ± 0.30a	4.59 ± 0.13ab	3.83 ± 0.11a
F value	5.25**	15.17**	11.84**	13.94**	6.55**	12.24**	9.51**	8.60**

WIN, water inorganic nitrogen; TN, total nitrogen; SS, seedling stage; FS, flowering stage; BS, boll-setting stage; BOS, boll-opening stage. Different treatments are defined in the Table 2. SS = Seedling stage, FS=Flowering stage, BS=Boll-setting stage, BOS=Boll-opening stage
F values and significance levels (**$P < 0.01$)

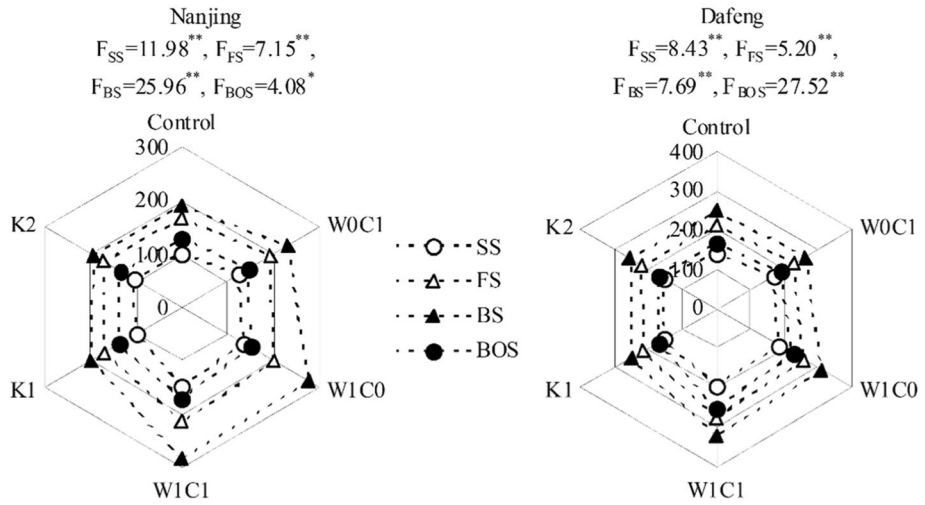

Fig. 2 Effects of crop residue incorporation and K fertilization on the amount of soil cellulose-decomposing bacteria ($\times 10^3 \cdot g^{-1}$ of CFU). Control, neither crop residue nor K fertilizer; W1C0, 0.9 t·ha^{-1} wheat straw incorporation alone, W0C1, 0.7 t·ha^{-1} cotton straw incorporation alone; W1C1, 0.9 t·ha^{-1} wheat straw incorporation + 0.7 t·ha^{-1} cotton straw incorporation; K150, 150 kg·ha^{-1} of K_2O; K300, 300 kg·ha^{-1} of K_2O. *F* values were given for SS, seedling stage; FS, flowering stage; BS, boll-setting stage and BOS, boll-opening stage. ** means $P < 0.01$; * means $P < 0.05$ and ^{ns} indicates $P \geq 0.05$

ratios ($P < 0.05$, Table 7). The activity of cellulase was significantly influenced by MBC/SOC, MBC/MBN and MBN/TN ratios ($P < 0.05$, Table 7). The β-glucosidase activity was significantly ($P < 0.05$, Table 7) impacted by MBC/SOC, WSOC/SOC, MBC/MBN and MBN/TN ratios. Moreover, the arylamidase activity was significantly influenced by MBN/TN, MBC/MBN and WSOC/SOC ratios ($P < 0.01$, Table 7). Obviously, MBC/MBN ratio was an important factor to influence the amount of cellulose-decomposing bacteria and the activities of cellulase, β-glucosidase and arylamidase.

Discussion

Effects of crop residue incorporation and inorganic K fertilization on soil C and N characteristics

A previous study reported that long-term K fertilization altered soil C and N characteristics (Belay et al. 2002). However, in the present study, compared with control, K fertilizer treatments had no significant effects on WSOC/SOC, MBC/SOC, WIN/TN, MBN/TN and MBC/MBN ratios, which were similar to the results reported by Kering et al. (2013) and Qiu et al. (2014). The different results between the previous long-term K fertilization

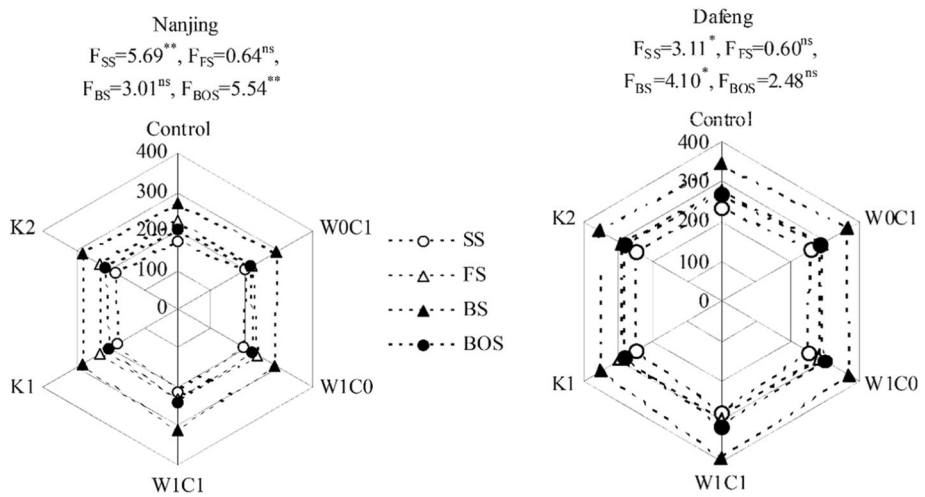

Fig. 3 Effects of crop residue incorporation and K fertilization on soil cellulase activity (mg·g^{-1}·(24 h^{-1}) of gulcose). Control, neither crop residue nor K fertilizer; W1C0, 0.9 t·ha^{-1} wheat straw incorporation alone, W0C1, 0.7 t·ha^{-1} cotton straw incorporation alone; W1C1, 0.9 t·ha^{-1} wheat straw incorporation + 0.7 t·ha^{-1} cotton straw incorporation; K150, 150 kg·ha^{-1} of K_2O; K300, 300 kg·ha^{-1} of K_2O. *F* values were given for SS, seedling stage; FS, flowering stage; BS, boll-setting stage and BOS, boll-opening stage. ** means $P < 0.01$; * means $P < 0.05$ and ^{ns} indicates $P \geq 0.05$

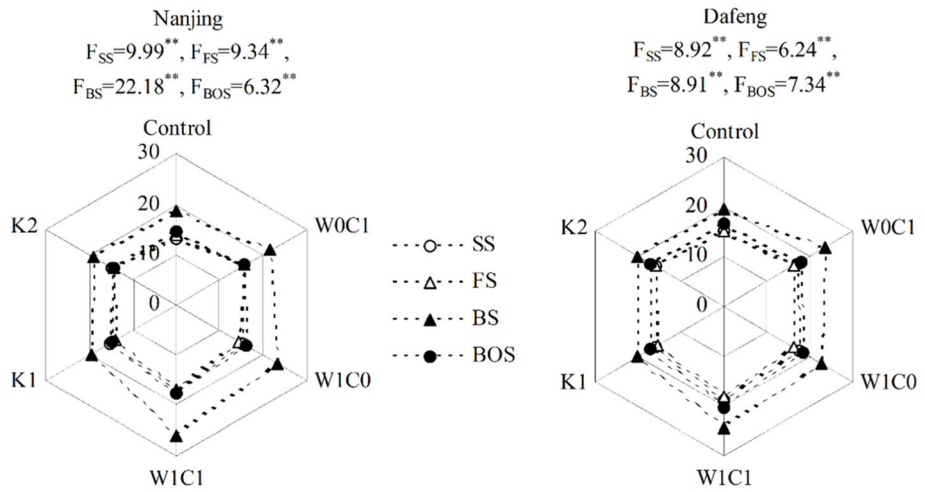

Fig. 4 Effects of residue incorporation and K fertilization on soil β-glucosidase activity (*p*-nitrophenol $g^{-1}·h^{-1}$). Control, neither crop residue nor K fertilizer; W1C0, 0.9 $t·ha^{-1}$ wheat straw incorporation alone, W0C1, 0.7 $t·ha^{-1}$ cotton straw incorporation alone; W1C1, 0.9 $t·ha^{-1}$ wheat straw incorporation + 0.7 $t·ha^{-1}$ cotton straw incorporation; K150, 150 $kg·ha^{-1}$of K_2O; K300, 300 $kg·ha^{-1}$ of K_2O. *F* values were given for SS, seedling stage; FS, flowering stage; BS, boll-setting stage and BOS, boll-opening stage. ** means $P < 0.01$; * means $P < 0.05$ and ^{ns} indicates $P \geq 0.05$

experiment and our experiment might be because that long-term K fertilizer application would result in loss of organic matter (Aref and Wander 1997) and alter related soil bacteria, actinomycetes and fungi amounts involved in soil C and N cycling (Belay et al. 2002), but short-term K fertilizer application had no effects on these parameters. Crop residue incorporation significantly increased WSOC/SOC, MBC/SOC, MBN/TN and MBC/MBN ratios. This should be because crop residue contains abundant C and N (Windeatt et al. 2014), and C and N cumulative release rates of crop residue were fast, with 48.29–66.55% and 48.35–67.49% within 90 days past incorporation, respectively (Wu et al. 2011). Although SOC and TN contents in soil would not be changed by short-term crop straw incorporation because of high background levels (Zhu et al. 2010), C and N released by crop residue would alter the chemical states of C and N in soil (Fig. 5).

Compared with control, WSOC/SOC and MBC/SOC ratios were not altered by W0C1 treatment (Tables 2 and 3), but WSOC/SOC ratio was increased by W1C0 and W1C1 treatments at least one growth stage (Table 4). Moreover, MBC/SOC ratio was also increased by W1C1 treatment, suggesting that the incorporation of

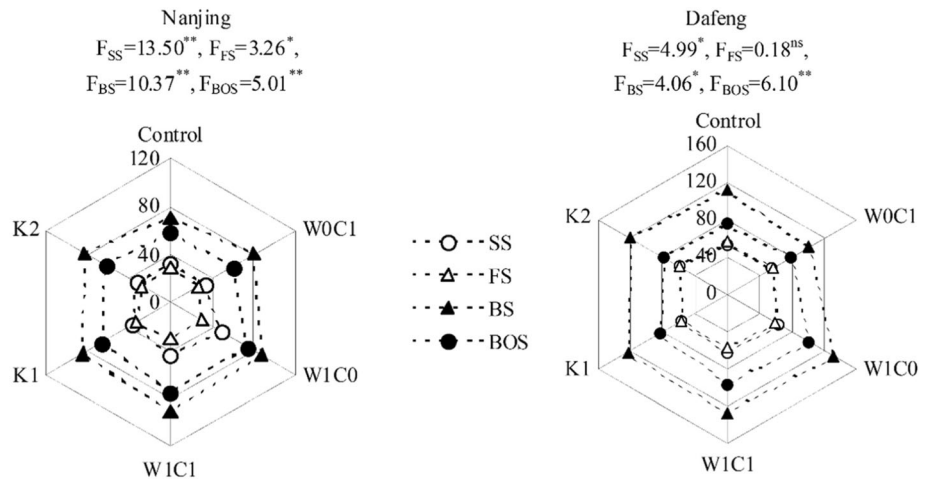

Fig. 5 Effects of crop residue incorporation and K fertilization on soil arylamidase activity (μg β-Naphthylamine $g^{-1}·h^{-1}$). Control, neither crop residue nor K fertilizer; W1C0, 0.9 $t·ha^{-1}$ wheat straw incorporation alone, W0C1, 0.7 $t·ha^{-1}$ cotton straw incorporation alone; W1C1, 0.9 $t·ha^{-1}$ wheat straw incorporation + 0.7 $t·ha^{-1}$ cotton straw incorporation; K150, 150 $kg·ha^{-1}$ of K_2O; K300, 300 $kg·ha^{-1}$ of K_2O. *F* values were given for SS, seedling stage; FS, flowering stage; BS, boll-setting stage and BOS, boll-opening stage. ** means $P < 0.01$; * means $P < 0.05$ and ^{ns} indicates $P \geq 0.05$

Table 5 Effects of crop residue incorporation and K fertilization on soil MBN/TN ratio (%)

Treatment	Nanjing				Dafeng			
	SS	FS	BS	BOS	SS	FS	BS	BOS
Control	6.00 ± 0.29a	7.76 ± 0.39a	10.78 ± 0.69a	7.23 ± 0.07a	6.67 ± 0.30a	7.78 ± 0.45ab	10.22 ± 0.22ab	8.34 ± 0.57a
W0C1	5.63 ± 0.19a	8.09 ± 0.30a	10.65 ± 0.90a	7.51 ± 0.21a	6.57 ± 0.34a	8.04 ± 0.36a	10.52 ± 0.20a	8.47 ± 0.48a
W1C0	5.63 ± 0.24a	6.44 ± 0.29b	8.95 ± 0.14b	6.75 ± 0.15b	6.30 ± 0.15a	7.26 ± 0.23bc	9.22 ± 0.40c	8.22 ± 0.49a
W1C1	5.72 ± 0.24a	6.45 ± 0.14b	9.98 ± 0.26ab	7.52 ± 0.19a	6.52 ± 0.16a	7.01 ± 0.36c	9.67 ± 0.41bc	8.30 ± 0.05a
K150	5.96 ± 0.25a	7.82 ± 0.05a	10.80 ± 0.63a	7.28 ± 0.34a	6.78 ± 0.33a	7.77 ± 0.17ab	10.28 ± 0.58ab	8.62 ± 0.32a
K300	6.00 ± 0.28a	7.76 ± 0.45a	10.84 ± 0.63a	7.23 ± 0.23a	6.71 ± 0.16a	8.01 ± 0.44a	10.45 ± 0.47a	8.45 ± 0.30a
F value	1.63	17.71**	4.65*	5.55**	1.39	4.44*	4.85*	0.38

MBN, microbial biomass nitrogen; TN, total nitrogen; SS, seedling stage; FS, flowering stage; BS, boll-setting stage; BOS, boll-opening stage. Different treatments are defined in the Table 2

F values and significance levels (**$P < 0.01$, *$P < 0.05$)

wheat straw was easier to affect soil C characteristics than the incorporation of cotton straw, and the effect of wheat straw combined with cotton straw incorporation was the most obvious. This might be because that compared with cotton straw, wheat straw has a higher percentage of C, and different structure between cotton straw and wheat straw results in that cotton straw needs a longer period than wheat straw to release C (Windeatt et al. 2014). Compared with control, a lower WIN/TN ratio was measured in W0C1, W1C0 and W1C1 treatments, and a higher MBN/TN ratio was observed in W1C0 and W1C1 treatments at multiple stages (Tables 5 and 6), suggesting that the incorporation of crop straw can easily affect the soil N cycle. Limon-Ortega et al. (2000) reported that the number of microorganism would increase during crop residues decomposition process, which consumed abundant soil WIN and increased MBN. This might be the reason why lower WIN/TN ratio and higher MBN/TN ratio were observed in crop straw incorporation treatments (apart from MBN/TN ratio in W0C1 treatment). However, the reasons why W0C1 had no effect on MBN/TN ratio need further exploration. MBC/MBN ratio was increased by W0C1, W1C0 and W1C1 treatments at least one growth stage, indicating that crop straw incorporation altered the biological activity of soil C and N (Wang et al. 2013).

Effects of crop residue incorporation and inorganic K fertilization on soil cellulose- decomposing bacteria amount and enzymes activities

Generally, cellulose can be directly degraded to cellobiose and glucose by soil cellulose-decomposing bacteria (Wyszkowska et al. 2007). In the present study, K fertilizer treatments did not influence the amount of cellulose-decomposing bacteria (Fig. 2), however, the treatments with crop residue incorporation (W0C1, W1C0 and W1C1) significantly increased the number of soil cellulose-decomposing bacteria, meaning that crop straw incorporation treatments had the potential to produce more cellulase. This was because that crop straw residues could change the pH of soil, and the altered pH might be good for the growth of beneficial bacteria, including cellulose-decomposing bacteria (Tayyab et al. 2018). In addition, straw incorporation could improve soil cellulose content which is the substrate for the cellulose-decomposing bacteria action. The increase of substrate stimulates the growth of cellulose-decomposing bacteria (Varga et al. 2004).

Table 6 Effects of crop residue incorporation and K fertilization on soil MBC/MBN ratio

Treatment	Nanjing				Dafeng			
	SS	FS	BS	BOS	SS	FS	BS	BOS
Control	5.46 ± 0.21b	5.65 ± 0.20b	5.11 ± 0.25b	5.30 ± 0.21ab	5.60 ± 0.27c	5.55 ± 0.25b	5.03 ± 0.27c	5.24 ± 0.23a
W0C1	5.95 ± 0.18a	5.51 ± 0.27b	5.26 ± 0.24b	5.39 ± 0.23ab	6.05 ± 0.28ab	5.79 ± 0.29b	5.17 ± 0.19bc	5.22 ± 0.25a
W1C0	5.62 ± 0.11b	6.65 ± 0.35a	5.87 ± 0.19a	5.56 ± 0.20ab	5.85 ± 0.21abc	6.30 ± 0.32a	5.53 ± 0.22ab	5.43 ± 0.22a
W1C1	6.06 ± 0.16a	7.02 ± 0.31a	6.09 ± 0.14a	5.73 ± 0.26a	6.11 ± 0.16a	6.61 ± 0.20a	5.81 ± 0.21a	5.58 ± 0.22a
K150	5.51 ± 0.19b	5.55 ± 0.23b	5.10 ± 0.26b	5.26 ± 0.20b	5.63 ± 0.22bc	5.87 ± 0.15b	5.10 ± 0.29c	5.20 ± 0.24a
K300	5.52 ± 0.18b	5.58 ± 0.12b	5.14 ± 0.22b	5.33 ± 0.21ab	5.70 ± 0.19abc	5.81 ± 0.14b	5.04 ± 0.14c	5.27 ± 0.21a
F value	6.55**	20.34**	11.79**	2.03	2.76	8.14**	6.15**	1.25

MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SS, seedling stage; FS, flowering stage; BS, boll-setting stage; BOS, boll-opening stage. Different treatments are defined in the Table 2

F values and significance levels (**$P < 0.01$)

Table 7 Effects of WSOC/SOC, MBC/SOC, WIN/TN, MBN/TN and MBC/MBN ratios on cellulose-decomposing bacteria amounts, cellulase, β-glucosidase and arylamidase activities analyzed by linear mixed models using the SAS software

Item	Cellulose-decomposing bacteria amount	Cellulase	β-glucosidase	Arylamidase
WSOC/SOC	4.128*	2.606	9.643**	4.065*
MBC/SOC	4.563*	6.099*	16.474**	0.149
WIN/TN	0.057	1.848	0.808	1.393
MBN/TN	2.421	4.257*	6.687*	8.977**
MBC/MBN	4.827*	4.500*	7.048**	4.159*

WSOC, water-soluble organic carbon content; SOC, soil organic carbon; MBC, microbial biomass carbon; WIN, water inorganic nitrogen; TN, total nitrogen; MBN, microbial biomass nitrogen; MBC, microbial biomass carbon
**$P < 0.01$, *$P < 0.05$

The cellulase can hydrolyze β-1,4-glucosidic bonds within the chains that comprise the cellulose polymer (Béguin and Aubert 1994; Bayer et al. 2006). The β-glucosidase activity is a limiting factor on accelerating the enzymatic conversion of cellulose, due to the removal of inhibitory levels of cellobiose (Sternberg et al. 1977). The two enzymes were correlated with C cycle between crop straw and soil. In the present study, K fertilizer treatments did not influence their activities (Figs. 3 and 4), but the treatments with crop residue incorporation (W0C1, W1C0 and W1C1) significantly increased activities of soil cellulase and β-glucosidase, suggesting that C cycle was accelerated in crops straw incorporation treatments. Allison and Killham (1988) and Varga et al. (2004) reported that increased soil organic matter and C contents which are conducive to bacterial active and enzymes activities in crop straw incorporation treatment might be the reason for increased activities of C cycle enzymes. Arylamidase as an initial limiting enzyme plays an important role in the N cycling in soils (Acosta-Martínez 2000). In this study, K fertilizer application and W0C1 treatments did not change its activity, nevertheless, W1C0 and W1C1 treatments significantly increased arylamidase activity, indicating that N might be more quickly released from wheat straw than cotton straw because of different structure between cotton straw and wheat straw (Windeatt et al. 2014) .

The results analyzed by mixed models showed that the amount of cellulose-decomposing bacteria and the activities of cellulase, β-glucosidase and arylamidase ($P < 0.05$, Table 7) can be affected by MBC/MBN ratio, indicating that MBC/MBN ratio was an important factor to influence soil bacteria and soil enzymes activities. MBC/MBN ratio is closely related to the proportion of microorganisms (Kara and Bolat 2008; Li et al. 2012) which will impact related soil enzymes activities. The treatments with crop residue incorporation significantly increased MBC/MBN ratio, which help to explain the phenomenon that crop residue incorporation treatments having a larger number of soil cellulose-decomposing bacteria and higher activities of soil enzymes.

Conclusion

Compared with control, short-term K fertilizer application had no effective influences on soil C and N characteristics, and soil microbial activities, however, crop residue incorporation promoted C and N cycle, and increased soil microbial activities, since W0C1, W1C0 and W1C1 treatments significantly increased WSOC/SOC, MBC/SOC and MBC/MBN ratios, and decreased WIN/TN ratio during cotton growth stages. W0C1, W1C0 and W1C1 treatments also increased the number of soil cellulose-decomposing bacteria and activities of cellulase, β-glucosidase and arylamidase. Moreover, compared with cotton straw incorporation treatment, wheat straw incorporation treatments had more obvious impacts on WSOC/SOC, MBC/SOC, MBC/MBN and WIN/TN ratios, the amount of soil cellulose-decomposing bacteria and activities of cellulase, β-glucosidase and arylamidase. Additionally, MBC/MBN ratio was the important factor leading to the differences in the amount of soil cellulose-decomposing bacteria and activities of soil enzymes among different treatments.

Abbreviations

BOS: Boll-opening stage; BS: Boll-setting stage; C: carbon; FS: Flowering stage; K: Potassium; MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen; N: Nitrogen; P: Phosphorus; SOC: Soil organic carbon; SS: Seedling stage; TN: Total nitrogen; WIN: Water inorganic nitrogen; WSOC: Water-soluble organic carbon content

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Authors' contributions

Conceived, designed and performed the experiments: Hu W, Sui N, Yu CR and Zhou ZG. Analyzed the data: Hu W and Sui N. Contributed reagents/materials/analysis tools: Yang CQ, Liu RX and Zhou ZG. Wrote the paper: Hu W and Sui N. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors have declared that no competing interests exist.

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